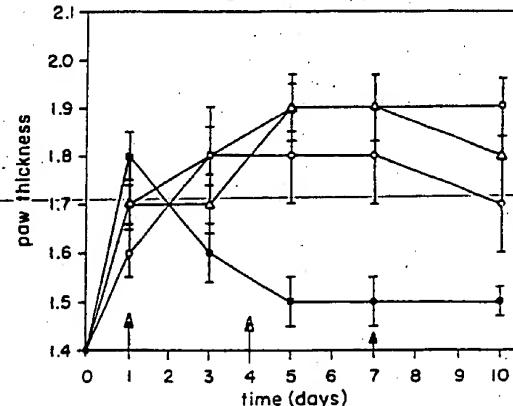
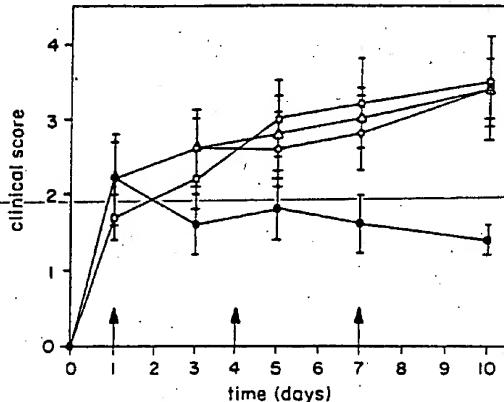




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<p>(21) International Application Number: <b>PCT/GB93/02070</b></p> <p>(22) International Filing Date: <b>6 October 1993 (06.10.93)</b></p> <p>(30) Priority data: <b>07/958,248 8 October 1992 (08.10.92) US</b></p> <p>(60) Parent Application or Grant (63) Related by Continuation US 07/958,248 (CIP) Filed on 8 October 1992 (08.10.92)</p> <p>(71) Applicant (for all designated States except US): <b>THE KENNEDY INSTITUTE OF RHEUMATOLOGY [GB/GB]; 6 Bute Gardens, Hammersmith, London W6 7DW (GB).</b></p>		<p>(72) Inventors; and (75) Inventors/Applicants (for US only) : <b>FELDMAN, Marc [AU/GB]; 2 Church Road, Hampstead, London N6 4QT (GB). MAINI, Ravinder, Nath [GB/GB]; 151 Castelnau, Barnes, London SW13 9EW (GB). WILLIAMS, Richard, Owen [GB/GB]; 60 Cheverell House, Pitchards Road, London E2 9BN (GB).</b></p> <p>(74) Agents: <b>HARVEY, David, Gareth et al.; Graham Watt &amp; Co., Riverhead, Sevenoaks, Kent TN13 2BN (GB).</b></p> <p>(81) Designated States: <b>AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b></p> <p><b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: TREATMENT OF AUTOIMMUNE AND INFLAMMATORY DISORDERS



## (57) Abstract

A method for treating autoimmune or inflammatory diseases, through the administration of anti-CD4 antibody in conjunction with or sequentially to anti-TNF antibody, is disclosed. The method can be used to aid in therapy for humans and other mammals with a wide variety of autoimmune or inflammatory diseases.

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TREATMENT OF AUTOIMMUNE AND INFLAMMATORY DISORDERS

Description

Background of the Invention

The nature of autoantigens responsible for autoimmune disorders is not known, nor is the action which triggers the autoimmune response. One popular theory involves the similarity of a viral protein to a self antigen, which results in autoreactive T cells or B cells recognizing a self antigen. Whereas B-lymphocytes produce antibodies, thymus-derived or "T-cells" are associated with cell-mediated immune functions. T-cells recognize antigens presented on the surface of cells and carry out their functions with these "antigen-presenting" cells.

Various markers have been used to define human T cell populations. CD4 is a non-polymorphic surface glycoprotein receptor with partial sequence identity to immunoglobulins. CD4 receptors define distinct subsets of mature peripheral T cells. In general, CD4 T cells expressing helper or regulatory functions interact with B cells in immune responses, while T cells expressing the CD8 surface antigen function as cytotoxic T cells and have regulatory effects on immune responses. Since T-cell receptors are the pathway through which stimuli augment or modulate T-cell responses, they present a potential target for immunological intervention.

Of the cellular interactions, that of CD4+ T cells with antigen presenting cells (APC) lies at the root of the immune response. Many aspects of the autoimmune response are essentially similar to that of normal immune responses. Thus CD4+ autoantigen reactive T cells are restimulated by APC expressing class II with autoantigen peptides in the binding groove. In certain human diseases the evidence that this occurs has been provided: in

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Graves' disease of the thyroid, in vivo activated T cells are present in the glands that are removed for refractory disease, and many of these cells after cloning can be shown to recognize autologous thyrocytes (as APC) not extrinsically supplied with any antigen, or APC supplied with the thyroid specific antigens thyroid peroxidase or thyroglobulin (Londei, M. et al., Science 228: 85-89 (1985); Dayan, C.M. et al., Proc. Natl. Acad. Sci. USA 88: 7415-7419 (1991)). Similarly, in rheumatoid arthritis (RA), in vivo activated T cells recognizing collagen type II have been isolated from joints of an RA patient in three consecutive operations during the course of three years (Londei, M. et al., Proc. Natl. Acad. Sci. 86: 636-640 (1989)). In other human diseases displaying autoimmune characteristics, CD4+ T cells from the blood have been cloned, including CD4+ cells recognizing the acetylcholine receptor in myasthenia gravis (Hohlfeld, R. et al., Nature 310: 224-246 (1984)); myelin basic protein in multiple sclerosis (Hafler, D.A. et al., J. Immunol. 139: 68-72 (1987)); or islet cell membranes in insulin dependent diabetes mellitus (De Berardinis, P. et al., Lancet II: 823-824 (1988); Kontiainen, S. et al., Autoimmunity 8: 193-197 (1991)).

Treatment with antibodies specific for CD4 is effective in preventing a wide range of both experimentally-induced and spontaneously-occurring autoimmune diseases. For example, treatment with either anti-CD4 or anti-MHC class II antibodies was found to effectively prevent murine collagen-induced arthritis as well as murine streptococcal cell wall-induced arthritis (Ranges, G.E. et al., J. Exp. Med. 162: 1105-1110 (1985); Hom, J.T. et al., Eur. J. Immunol. 18: 881-888 (1988); Wooley, P.H. et al., J. Immunol. 134: 2366-2374 (1985);

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Cooper, S.M. et al., J. Immunol. 141: 1958-1962 (1988); Van den Broek, M.F. et al., Eur. J. Immunol. 22: 57-61 (1992)). Anti-CD4 treatment also prevented systemic lupus erythematosus in NZB/NZW F1 (B/W) mice and BXSB mice  
5 (Wofsy, D. et al., J. Immunol. 134: 852-857 (1985); Wofsy, D. et al., J. Immunol. 136: 4554-4560 (1986); Ermak, T.J. et al., Laboratory Investigation 61: 447-456 (1989)). Anti-T cell/APC treatment is less effective, or completely ineffective, however, in reducing the severity of  
10 established disease (i.e., after onset). For example, neither anti-CD4 nor anti-MHC class II treatment was effective in reducing the severity of established collagen-induced arthritis in mice (Hom, J.T. et al., Eur. J. Immunol. 18: 881-888 (1988); Cooper, S.M. et al., J. Immunol. 141: 1958-1962 (1988)). For practical therapy in humans, it is treatment after the onset of disease which is needed, as well as prevention. Hence there is evidence  
15 that blocking the CD4 T cell/APC interaction by itself may not be an optimal mode of therapy, and that it is possible  
20 that its efficacy may be augmented by other means.

Factors other than CD4 also influence cellular immune response. The cytokine tumor necrosis factor- $\alpha$  (TNF $\alpha$ ; also termed cachectin) has multiple effects on inflammation, tissue damage, immune response and cell  
25 trafficking into lesions, and thus plays a role in the pathogenesis of inflammatory joint diseases, including rheumatoid arthritis (Brennan, F.M. et al., Lancet ii, 244-247 (1989); Feldmann, M. et al., Ann. Rheumatic Dis. 51: 480-486 (1990)). TNF $\alpha$  is a protein secreted primarily  
30 by monocytes and macrophages in response to endotoxin or other stimuli as a soluble homotrimer of 17 kD protein subunits (Smith, R.A. et al., J. Biol. Chem. 262: 6951-6954 (1987)). A membrane-bound 26 kD precursor form of

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TNF has also been described (Kriegler, M. et al., Cell **53**: 45-53 (1988)). The expression of the gene encoding TNF $\alpha$  is not limited to cells of the monocyte/macrophage family: TNF is also produced by CD4+ and CD8+ peripheral blood T lymphocytes, and by various cultured T and B cell lines (Cuturi, M.C. et al., J. Exp. Med. **165**: 1581 (1987); Sung, S.-S.J. et al., J. Exp. Med. **168**: 1539 (1988); Turner, M. et al., Eur. J. Immunol. **17**: 1807-1814 (1987)). Recent evidence implicates TNF in the autoimmune pathologies and graft versus host pathology (Piguet, P.-F. et al., J. Exp. Med. **166**: 1280 (1987)). It has been demonstrated that the hamster anti-TNF monoclonal antibody TN3.19.2, which reacts with TNF $\alpha$  and may also react with TNF $\beta$ , can markedly diminish the severity of joint destruction and reduce inflammation in collagen type II induced arthritis in the DBA/1 mouse, irrespective of whether treatment was started before or after the onset of arthritis (Williams, R.O. et al., Proc. Natl. Acad. Sci. USA **89**:9784-9788 (1992)). However, anti-TNF therapy did not completely eliminate arthritis, suggesting that factors other than TNF contribute to the pathology.

Despite these and other advances, a great need remains for better therapies for autoimmune and inflammatory diseases.

**25 Summary of the Invention**

The current invention pertains to the discovery that combination therapy, involving the use of anti-CD4 antibodies in conjunction with anti-TNF antibodies, produces markedly superior results than the use of each agent alone in the treatment of autoimmune or inflammatory disease, particularly in rheumatoid arthritis. Anti-CD4 antibodies are administered to the subject simultaneously

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or sequentially with anti-TNF antibodies. The antibodies can be administered together with a pharmaceutically acceptable vehicle; administration can be in the form of a single dose, or a series of doses separated by intervals 5 of days or weeks.

Combination therapy can also utilize an agent other than anti-CD4 antibody which affects the activation or interaction of CD4+ cells with antigen presenting cells, in combination with an inflammatory mediator other than 10 anti-TNF antibodies.

The benefits of combination therapy with anti-CD4 antibody and anti-TNF antibody include improved results in comparison with the effects of treatment with each therapeutic modality separately. In addition, lower 15 dosages can be used to provide the same reduction of the immune and inflammatory response, thus increasing the therapeutic window between a therapeutic and a toxic effect. Lower doses may also result in lower financial costs to the patient, and potentially fewer side effects.

20 Brief Description of the Figures

Figure 1 contains a set of graphs, individually labelled as Fig. 1A and Fig. 1B, from an experiment which illustrates the suppression of arthritis as assessed by clinical score (Fig. 1A) and pawswelling measurements 25 (Fig. 1B) after the administration of 50 µg anti-TNF (hamster TN3.19.2) and 200 µg anti-CD4 to DBA/1 male mice. Open squares = control; diamonds = anti-CD4; triangles = anti-TNF (50 µg); closed squares = anti-CD4/anti-TNF (50 µg).

Figure 2 contains a set of graphs, individually 30 labelled as Fig. 2A, Fig. 2B, Fig. 2C, and Fig. 2D, from a second experiment which illustrates the potentiation of anti-CD4 with low dose (50 µg) anti-TNF or high dose (300

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- μg) anti-TNF on clinical score and paws swelling measurements. Fig. 2A: clinical score with low-dose anti-TNF; Fig. 2B: clinical score with high-dose anti-TNF; Fig. 2C: paws swelling with low-dose anti-TNF; Fig. 5 2D: paws swelling with high-dose anti-TNF. Open squares = control; diamonds = anti-CD4; triangles = anti-TNF (50μg); closed squares = anti-CD4/anti-TNF (50μg).

Detailed Description of the Invention

- The present invention concerns the treatment of  
10 autoimmune or inflammatory diseases, such as rheumatoid arthritis, through the administration of anti-CD4 antibody in conjunction with anti-TNF antibody. The term antibody is intended to encompass both polyclonal and monoclonal antibodies. The term antibody is also intended to  
15 encompass mixtures of more than one antibody reactive with CD4 or with TNF (e.g., a cocktail of different types of monoclonal antibodies reactive with CD4 or with TNF). The term antibody is further intended to encompass whole antibodies, biologically functional fragments thereof, and  
20 chimeric antibodies comprising portions from more than one species, bifunctional antibodies, etc. Biologically functional antibody fragments which can be used are those fragments sufficient for binding of the antibody fragment to CD4 or to TNF.
- 
- 25 The chimeric antibodies can comprise portions derived from two different species (e.g., human constant region and murine variable or binding region). The portions derived from two different species can be joined together chemically by conventional techniques or can be prepared  
30 as single contiguous proteins using genetic engineering techniques. DNA encoding the proteins of both the light

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chain and heavy chain portions of the chimeric antibody can be expressed as contiguous proteins.

Monoclonal antibodies reactive with CD4 or with TNF can be produced using somatic cell hybridization techniques (Kohler and Milstein, Nature 256: 495-497 (1975)) or other techniques. In a typical hybridization procedure, a crude or purified protein or peptide comprising at least a portion of CD4 or of TNF can be used as the immunogen. An animal is vaccinated with the immunogen to obtain anti-CD4 or anti-TNF antibody-producing spleen cells. The species of animal immunized will vary depending on the species of monoclonal antibody desired. The antibody producing cell is fused with an immortalizing cell (e.g., myeloma cell) to create a hybridoma capable of secreting anti-CD4 or anti-TNF antibodies. The unfused residual antibody-producing cells and immortalizing cells are eliminated. Hybridomas producing desired antibodies are selected using conventional techniques and the selected hybridomas are cloned and cultured.

Polyclonal antibodies can be prepared by immunizing an animal with a crude or purified protein or peptide comprising at least a portion of CD4 or of TNF. The animal is maintained under conditions whereby antibodies reactive with either CD4 or TNF are produced. Blood is collected from the animal upon reaching a desired titer of antibodies. The serum containing the polyclonal antibodies (antisera) is separated from the other blood components. The polyclonal antibody-containing serum can optionally be further separated into fractions of particular types of antibodies (e.g., IgG, IgM).

A more detailed description of anti-CD4 antibodies and their use in treatment of disease is contained in the

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- following references, the teachings of which are hence incorporated by reference: U.S. Application No. 07/867,100, filed June 25, 1992; Grayheb, J. et al., J. of Autoimmunity 2:627-642 (1989); Ranges, G.E. et al., J. Exp. Med. 162: 1105-1110 (1985); Hom, J.T. et al., Eur. J. Immunol. 18: 881-888 (1988); Wooley, P.H. et al., J. Immunol. 134: 2366-2374 (1985); Cooper, S.M. et al., J. Immunol. 141: 1958-1962 (1988); Van den Broek, M.F. et al., Eur. J. Immunol. 22: 57-61 (1992); Wofsy, D. et al., J. Immunol. 134: 852-857 (1985); Wofsy, D. et al., J. Immunol. 136: 4554-4560 (1986); Ermak, T.J. et al., Laboratory Investigation 61: 447-456 (1989); Reiter, C. et al., 34:525-532 (1991); Herzog, C. et al., J. Autoimmun. 2:627 (1989); Ouyang, Q. et al., Dig. Dis. Sci. 33:1528-1536 (1988); Hertzog, C. et al., Lancet, p. 1461 (December 19, 1987); Emmrich, J. et al., Lancet 338:570-571 (August 31, 1991).

- A more detailed description of anti-TNF antibodies and their use in treatment of disease is contained in the following references, the teachings of which are hence incorporated by reference: U.S. Application No. 07/943,852, filed September 11, 1992; Rubin et al., (EPO Patent-Publication-0218868,-April-22,-1987); Yone et al., (EPO Patent Publication 0288088, October 26, 1988); Liang, C.-M. et al., Biochem. Biophys. Res. Comm. 137:847-854 (1986); Meager, A. et al., Hybridoma 6:305-311 (1987); Fendly et al., Hybridoma 6:359-369 (1987); Bringman, T.S. et al., Hybridoma 6:489-507 (1987); Bringman T.S. et al., Hybridoma 6:489-507 (1987); Hirai, M. et al., J. Immunol. 96:57-62 (1987); Moller, A. et al., Cytokine 2:162-169 (1990); Mathison, J.C. et al., J. Clin. Invest. 81:1925-1937 (1988); Beutler, B. et al., Science 229:869-871 (1985); Tracey, K.J. et al., Nature 330:662-664

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- (1987); Shimamoto, Y. et al., Immunol. Lett. 17:311-318  
(1988); Silva, A.T. et al., J. Infect. Dis. 162: 421-427  
(1990); Opal, S.M. et al., J. Infect. Dis. 161:1148-1152  
(1990); Hinshaw, L.B. et al., Circ. Shock 30:279-292  
5 (1990).

- The antibodies can be administered subcutaneously, intravenously, intramuscularly, topically, orally, rectally, nasally, buccally, vaginally, by inhalation spray, or via an implanted reservoir in dosage  
10 formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The form in which the antibodies are administered (e.g., capsule, tablet, solution, emulsion) will depend at least in part on the route by which it is  
15 administered.

A therapeutically effective amount of the combination of anti-CD4 antibody and anti-TNF antibody is that amount necessary to significantly reduce or eliminate symptoms associated with a particular autoimmune or inflammatory disorder. The preferred therapeutically effective amount is in the range of 0.1 - 10 mg/kg/dose of each antibody. The therapeutically effective amount will be determined on an individual basis and will be based, at least in part, on consideration of the individual's size, the severity of  
20 symptoms to be treated, the result sought, etc. Thus, the therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

- The therapeutically effective amount can be  
30 administered in the form of a single dose, or a series of doses separated by intervals of days or weeks. Once the therapeutically effective amount has been administered, a maintenance amount of anti-CD4, of anti-TNF, or of a

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combination of anti-CD4 and anti-TNF can be administered. A maintenance amount is the amount of anti-CD4, anti-TNF, or combination of anti-CD4 and anti-TNF necessary to maintain the reduction or elimination of symptoms achieved by the therapeutically effective dose. The maintenance amount can be administered in the form of a single dose, or a series of doses separated by intervals of days or weeks. Like the therapeutically effective amount, the maintenance amount will be determined on an individual basis.

Other anti-inflammatory drugs, such as the anti-rheumatic drugs methotrexate or cyclosporin A, can be administered in conjunction with the anti-CD4 antibody or the anti-TNF antibody.

Although the experimental data discussed herein involves the combination of anti-CD4 antibody and anti-TNF antibody, combination therapy involving the use of an agent, other than or in addition to anti-CD4 antibodies, which affects the activation or interaction of CD4+ cells with antigen presenting cells (APC), in combination with an inflammatory mediator, other than or in addition to anti-TNF antibodies, can also be used to treat autoimmune or inflammatory diseases.

The CD4+ affecting agent can include antibodies to T cells or their receptors, such as anti-CD4, anti-CD28, anti-CD52 (e.g., CAMPATH-1H) and anti-IL-2R; antibodies to APC or their receptors, such as anti-class II, anti-ICAM-1, anti-LFA-3, and anti-LFA-1; peptides and small molecules blocking the T cell/APC interaction, including those which block the HLA class II groove, or block signal transduction in T-cell activation, such as cyclosporins or FK-506; and antibodies to B cells including CD5+ B cells, such as CD19, 20, 21, 23 and BB/7 or B1, ligands for CD28,

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B cells including CD5+ B cells are considered to be an important type of APC in disease processes (Plater-Zyberk, C. et al., Ann. N.Y. Acad. Sci. 651: 540-555 (1992)), and thus anti-B cell antibodies can be particularly useful in 5 the current invention.

The inflammatory mediators can include agents interfering with TNF, such as anti-TNF antibody, soluble TNF-R (monomeric, IgG fusion proteins, etc.), or blocking peptides and small molecules interfering with TNF receptor 10 signalling or with TNF synthesis, such as pentoxyfilline and thalidomide; agents interfering with IL-1, such as anti-IL-1 antibody, soluble IL-1R, IL-1 receptor antagonist, or blocking peptides and small molecules influencing IL-1 synthesis or IL-1 receptor signalling; 15 agents interfering with IL-6, such as anti-IL-6 antibody, anti-gp 130, or blocking peptides and small molecules affecting synthesis or receptor signalling of IL-6; modalities influencing other inflammatory mediators, such as GM-CSF and members of the chemokine (IL-8) family; and 20 cytokines with anti-inflammatory properties, such as IL-4, IL-10, and TGF $\beta$ .

The combination therapy of the current invention is thus useful for the treatment of many autoimmune or inflammatory diseases of humans and of animals. In 25 humans, diseases for which the therapy is appropriate include rheumatoid arthritis (RA) and juvenile chronic arthritis (JCA). Other diseases and conditions for which combination therapy is appropriate include spondyloarthropathies, such as ankylosing spondylitis, 30 psoriatic arthritis, or arthritis associated with inflammatory bowel disease; vasculitides, such as polyarteritis nodosa, Wegener's granulomatosis, giant cell arteritis, Henoch-Schoenlein purpura, and microscopic

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vasculitis of the kidneys; Sjogren's syndrome; systemic lupus erythematosus; inflammatory bowel disease, including Crohn's disease and ulcerative colitis; chronic active hepatitis; primary biliary cirrhosis; cryptogenic

- 5 fibrosing alveolitis and other fibrotic lung diseases; uveitis; multiple sclerosis; myasthenia gravis; hemolytic anemia; scleroderma; graft versus host disease; allergy; and transplantation of kidneys, liver, heart, lungs, bone marrow, skin, or of other organs.

10 The invention is further and more specifically illustrated by the following exemplification.

EXEMPLIFICATION: Treatment of Induced Arthritis in a Murine Model.

15 The murine model of collagen type II induced arthritis has similarities to rheumatoid arthritis (RA) in its marked MHC class II predisposition, as well as in histology, immunohistology, erosions of cartilage and bone, and in its response to anti-TNF therapy. Thus the

20 animal model serves as a good approximation to human disease. The model of rheumatoid arthritis used herein is described by Williams, R.O. et al., (PNAS, 89:9784-9788 (1992), i.e., the collagen type II induced arthritis in the DBA/1 mouse. Type II collagen was purified from

25 bovine articular cartilage by limited pepsin solubilization and salt fractionation as described by Miller (Biochemistry 11:4903-4909 (1972)).

Study 1

30 DBA/1 male mice were immunized intradermally at 8-12 weeks of age with 100 µg of bovine type II collagen emulsified in complete Freund's adjuvant, and 21 days later with 100 µg of collagen intra-peritoneally (i.p.).

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Immediately after the onset of clinically evident arthritis (redness and/or swelling in one or more limbs), which was about 35 days after the initial injection, mice were injected i.p. with anti-CD4; anti-TNF; anti-CD4 and 5 anti-TNF; or isotype controls. Arthritis was monitored for clinical score and paw-swelling for 10 days. Antibody treatment was administered on day 1 (onset), day 4 and day 7.

Two experiments were completed, assessing clinical 10 score and paws swelling. In each, 200 µg of anti-CD4 were used per injection (rat YTS 191 and YTA 3.1) was used. Clinical score was assessed on the following scale: 0 = normal; 1 = slight swelling and/or erythema; 2 = pronounced edematoma swelling; and 3 = joint rigidity.

15 Each limb was graded, giving a maximum score of 12 per mouse. Paws swelling was monitored by measuring the thickness of each affected hind paw with calipers. The results were expressed as the percentage increment in paw width relative to the paw width before the onset of 20 arthritis.

In the first experiment, a single dose of 50 µg per injection of anti-TNF (hamster TN3.19.2) was administered to each of five mice per group. There was no significant effect of anti-CD4 or anti-TNF (TN3.19 given 3 times at 25 50 µg/mouse). Hence the benefit of combination therapy, in both clinical score and footpad swelling, is readily seen (see Figures 1A, 1B).

In the second experiment, either 50 µg or 300 µg of anti-TNF were administered to each of 7 mice per group. 30 Both anti-CD4 and anti-TNF at low (50 µg) concentration had some effect, and benefit of combination therapy of these two concentrations was noted in paws swelling, not in clinical score. However, if anti-TNF was injected at

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300 µg/mouse, the benefit of combination therapy with anti-CD4 was seen in both clinical score and more clearly in paw-swelling (see Figures 2A, 2B, 2C, 2D).

The results of the experiments indicate that there is  
5 a clear benefit to combination therapy with anti-TNF and  
anti-CD4 antibodies, as measured by clinical score and  
foot pad swelling.

Study 2

Male DBA/1 mice were immunized intradermally at 8-12  
10 weeks of age with 100 µg type II collagen emulsified in  
Freund's complete adjuvant. Day one of arthritis was  
considered to be the day that erythema and/or swelling was  
first observed in one or more limbs. Arthritis became  
clinically evident around 30 days after immunization with  
15 type II collagen. For each mouse, treatment was started  
on the first day that arthritis was observed and continued  
over a 10 day period, after which the mice were sacrificed  
and joints were processed for histology. Monoclonal  
antibody treatment was administered on days 1, 4, and 7.  
20 First, a sub-optimal dose of 50 µg of anti-TNF alone (TN3-  
19.12, hamster IgG1 anti-TNF $\alpha/\beta$  mAb) was compared with the  
same dose given together with 200 µg of anti-CD4 (rat  
IgG2b, a mixture of YTS 191.1.2 and YTA 3.1.2). To verify  
the results, two separate but identical experiments were  
25 carried out (11-12 mice/group and 7-8 mice/group,  
respectively). Neither anti-CD4 alone nor sub-optimal  
anti-TNF alone were able to significantly reduce paw-  
swelling (data not shown). However, treatment with anti-  
TNF and anti-CD4 resulted in a consistently and  
30 statistically significant reduction in paw-swelling  
relative to the group given control mAb ( $P < 0.001$ ).  
Furthermore, in both experiments, combined anti-TNF/anti-

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CD4 treatment (also referred to herein as anti-CD4/TNF treatment) produced a significant reduction in paw-swelling relative to anti-CD4 alone, and anti-TNF alone ( $P < 0.05$ ).

- 5 Next, an optimal dose of anti-TNF (300 µg) alone was compared in two separate but identical experiments (7-7 mice/group and 6-7 mice/group, respectively) with the same dose given in combination with anti-CD4. As before, the combined anti-TNF/anti-CD4 treatment resulted in a  
10 significant reduction in paw-swelling compared to treatment with the control mAb ( $P < 0.005$ ; data not shown). In the first experiment, paw-swelling was also significantly reduced in the combined anti-CD4/anti-TNF treated group relative to the groups given anti-CD4 alone  
15 or anti-TNF alone ( $P < 0.05$ ). Some reduction in paw-swelling was observed in mice given either anti-TNF alone or anti-CD4 alone although the differences were not significant, possibly because of the small group sizes (6 per group). In the second experiment, combined anti-  
20 CD4/anti-TNF gave significantly reduced paw-swelling compared to anti-CD4 alone ( $P < 0.05$ ) but not compared to anti-TNF alone since anti-TNF itself caused a significant reduction in paw-swelling, as expected from previous work (Williams, R.O. et al., PNAS 89: 9784-9788 (1992)). In  
25 the experiments, the reduction in paw-swelling attributable to anti-TNF alone was 23% and 33%, respectively. Thus, the reduction in paw-swelling attributable to anti-TNF treatment was broadly comparable with our previously published findings in which treatment  
30 with TN3-119.12 (300 µg/mouse) resulted in a mean reduction in paw-swelling over the treatment period of around 34% relative to controls (Williams, R.O. et al., PNAS 89: 9784-9788 (1992)).

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Limb Involvement

In collagen-induced arthritis, as in RA, it is usual for additional limbs to become involved after the initial appearance of clinical disease and new limb involvement is  
5 an important indicator of the progression of the disease.  
To determine the effect of anti-CD4/anti-TNF treatment on new limb involvement, the number of limbs with clinically detectable arthritis at the end of the 10 day treatment period was compared with the number of arthritis limbs  
10 before treatment. In mice given the control mAb there was an increase in limb involvement over the 10 day period of approximately 50%. The results from the two experiments were pooled, and are shown in Table 1.

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**Table 1: Combined anti-CD4/anti-TNF Inhibits Progression of Clinical Arthritis**

	Treatment	Number of Limbs Affected (Mean $\pm$ SEM)		Increase (%)
		Day 1	Day 10	
<b>Sub-optimal anti-TNF (50 µg)</b>				
5	anti-CD4 (n=18)	1.30 $\pm$ 0.10	1.90 $\pm$ 0.13	46.1
	anti-TNF (n=19)	1.20 $\pm$ 0.09	1.65 $\pm$ 0.17	37.5
	anti-CD4/TNF (n=18)	1.40 $\pm$ 0.09	1.45 $\pm$ 0.22	3.4 <sup>1</sup>
	control mAb (n=18)	1.43 $\pm$ 0.15	2.24 $\pm$ 0.18	56.6
<b>Optimal anti-TNF (300 µg)</b>				
10	anti-CD4 (n=12)	1.27 $\pm$ 0.10	1.80 $\pm$ 0.14	42.0
	anti-TNF (n=11)	1.50 $\pm$ 0.17	1.64 $\pm$ 0.20	9.5 <sup>2</sup>
	anti-CD4/TNF (n=13)	1.25 $\pm$ 0.11	1.25 $\pm$ 0.11	0 <sup>3</sup>
15	control mAb (n=12)	1.53 $\pm$ 0.19	2.27 $\pm$ 0.25	47.8

1 P < 0.05 (anti-CD4/TNF vs. control mAb)

2 P < 0.05 (anti-TNF vs. control mAb)

3 P < 0.005 (anti-CD4/TNF vs. control mAb)

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There was some reduction in new limb involvement in the groups given anti-CD4 alone and sub-optimal anti-TNF alone, although the differences were not significant. In the group given optimal anti-TNF the increase in limb involvement was less than 10% ( $P < 0.05$ ). More striking, however, was the almost complete absence of new limb involvement in the groups given combined anti-CD4/anti-TNF. Thus, the increase in new limb involvement was only 3% in mice given anti-CD4 plus suboptimal anti-TNF (10 ( $P < 0.05$ ) and 0% in mice given anti-CD4 plus optimal anti-TNF ( $P < 0.005$ ).

Histology

After 10 days, the mice were sacrificed; the first limb that had shown clinical evidence of arthritis was removed from each mouse, formalin-fixed, decalcified, and wax-embedded before sectioning and staining with haematoxylin and eosin. A sagittal section of the proximal interphalangeal (PIP) joint of the middle digit was studied in a blind fashion for the presence or absence of erosions in either cartilage or bone (defined as demarcated defects in cartilage or bone filled with inflammatory tissue). The comparisons were made only between the same joints, and the arthritis was of identical duration. Erosions were observed in almost 100% of the PIP joints from the control groups and in approximately 70-80% of the joints given either anti-CD4 alone or sub-optimal anti-TNF alone. The results of the two experiments were pooled, and are shown in Table 2.

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**Table 2:** Proportions of PIP Joints Showing Significant Erosion of Cartilage and/or Bone

Treatment	Joints with Erosions
Sub-optimal anti-TNF (50 µg)	
anti-CD4	13/18 (72%)
anti-TNF	14/19 (74%)
anti-CD4/TNF	4/18 (22%) <sup>1</sup>
control mAb	17/18 (94%)
Optimal anti-TNF (300 µg)	
anti-CD4	10/12 (83%)
anti-TNF	6/11 (54%) <sup>2</sup>
anti-CD4/TNF	4/13 (31%) <sup>3</sup>
control mAb	12/12 (100%)

- 15 1 P < 0.01 (anti-CD4/TNF vs. anti-CD4 alone; anti-TNF  
alone and control mAb)  
2 P < 0.01 (anti-TNF alone vs. control mAb)  
3 P < 0.01 (anti-CD4/TNF vs. anti-CD4 alone and control  
mAb)

20 An optimal dose of anti-TNF alone significantly  
reduced pathology, as reported previously (Williams, R.O.  
et-al., PNAS 89: 9784-9788 (1992)). Thus, in the mice  
given optimal anti-TNF alone the proportion of joints  
showing erosive changes was reduced to 54% (P < 0.001)  
25 whereas in the groups given anti-CD4 plus either sub-  
optimal or optimal anti-TNF, only 22% (P < 0.01) and 31%  
(P > 0.01) of the joints, respectively, were eroded.  
Thus, 300 µg of anti-TNF alone gave a degree of protection  
against joint erosion but combined anti-CD4/anti-TNF  
30 provided significantly greater protection.

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Depletion of CD4+ T Cells

The extent to which anti-CD4 treatment depleted peripheral CD4+ T cells was determined by flow cytometry. To enumerate the proportion of CD4+ lymphocytes in 5 disassociated spleen populations or peripheral blood, cells were incubated with phycoerythrin-conjugated anti-CD4 (Becton Dickinson, Oxford, UK), then analyzed by flow cytometry (FACScan, Becton Dickinson) with scatter gates set on the lymphocyte fraction. Anti-CD4 treatment 10 resulted in 98% ( $\pm$  1%) depletion of CD4+ cells in the spleen and 96% ( $\pm$  3%) depletion of CD4+ T cells in the blood.

Immunohistochemistry

The possible persistence of CD4+ T cells in the joint 15 despite virtual elimination of peripheral CD4+ T cells was next investigated by immunohistochemical analysis of sections from treated arthritic mice. Wax-embedded sections were de-waxed, trypsin digested, then incubated with anti-CD4 mAb (YTS 191.1.2/YTA 3.1.2). To confirm the 20 T cell identity of the CD4+ cells, sequential sections were stained with anti-Thy-1 mAb (YTS 154.7) (Cobbold, S.P. et al., Nature 312:548-551 (1984)). Control sections were incubated with HRPN11/12a. Detection of bound antibody was by alkaline phosphatase/rat anti-alkaline 25 phosphatase complex (APAAP; Dako, High Wycombe, UK) and fast red substrate as described (Deleuran, B.W. et al., Arthritis & Rheumatism 34:1125-1132 (1991)). Small numbers of CD4+ cells were detected in the joints, not only of mice given control mAb, but also of those treated 30 with anti-CD4 (data not shown). Furthermore, within the small number of mice that were studied (four per treatment group), it was not possible to detect significantly reduced

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numbers of CD4+ T cells in the groups given anti-CD4 alone or anti-CD4 plus anti-TNF (data not shown). Anti-CD4 treatment did not, therefore, eliminate CD4+ T cells from the joint.

5        Anti-collagen IgG Levels

Serum anti-collagen IgG levels were measured by enzyme-linked immunosorbent assay (ELISA). Microtitre plates were coated with bovine type II collagen (2 µg/ml), blocked, then incubated with test sera in serial dilution 10 steps. Detection of bound IgG was by incubation with alkaline phosphatase-conjugated goat anti-mouse IgG, followed by substrate (dinitrophenyl phosphate). Optical densities were read at 405 nm. A reference sample, consisting of affinity-purified mouse anti-type II 15 collagen antibody, was included on each plate. Serum levels of anti-type II collagen IgG were not significantly altered within the 10 day treatment period by anti-CD4 alone, anti-TNF alone, or anti-CD4 plus anti-TNF (Table 3).

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**Table 3: Serum Levels of Anti-type II collagen IgG**

Treatment		Anti-collagen IgG (Mean ± SEM) (µg/ml)
<b>Sub-optimal anti-TNF (50 µg)</b>		
	anti-CD4 (n=18)	285 ± 37
5	anti-TNF (n=19)	208 ± 29
	anti-CD4/TNF (n=18)	208 ± 34
	control mAb (n=18)	238 ± 36
<b>Optimal anti-TNF (300 µg)</b>		
10	anti-CD4 (n=12)	288 ± 39
	anti-TNF (n=11)	315 ± 49
	anti-CD4/TNF (n=13)	203 ± 33
	control mAb (n=12)	262 ± 47

15        Anti-globulin Response

To find out whether anti-CD4 treatment prevented a neutralizing anti-globulin response against the anti-TNF mAb, IgM anti-TN4-19.12 levels on day 10, as measured by ELISA, were compared. At this time, an IgG anti-TN3-19.12 response was not detected. Microtitre plates were coated with TN3-19.12 (5 µg/ml), blocked, then incubated with serially diluted test sera. Bound IgM was detected by goat anti-mouse IgM-alkaline phosphatase conjugate, followed by substrate. The results demonstrated that anti-CD4 was highly effective in preventing the development of an anti-TN3-19.12 antibody response (Table 4). Next, to determine whether anti-CD4 treatment led to increased levels of circulating anti-TNF- $\alpha$  (by reducing the antibody response to the hamster anti-TNF), an ELISA

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was carried out in which recombinant murine TNF- $\alpha$  was used to detect free TN3-19.12 in the sera of mice on day 10 of the experiment. Microtitre plates were coated with recombinant murine TNF- $\alpha$ , blocked, then incubated with 5 test sera. Goat anti-hamster IgG-alkaline phosphatase conjugate (adsorbed against murine IgG) was then applied, followed by substrate. Quantitation was by reference to a sample of known concentration of TN3-19.12. Levels of TN3-19.12 were slightly elevated in the groups given anti-10 CD4 plus anti-TNF compared to anti-TNF alone, although the differences were not significantly different (Table 4).

**Table 4: IgM anti-TN3 Titres and Levels of Unbound TN3**

	Treatment	Reciprocal	Unbound TN3
		of Anti-TN3 Titre (Mean)	(Mean $\pm$ SEM) ( $\mu$ g/ml)
<b>Sub-optimal anti-TNF (50 <math>\mu</math>g)</b>			
	anti-TNF (n = 12)	242	8.6 $\pm$ 2.0
	anti-CD4/TNF (n = 12)	84 <sup>1</sup>	12.1 $\pm$ 1.9
	<b>Optimal anti-TNF (300 <math>\mu</math>g)</b>		
	anti-TNF (n = 12)	528	90.7 $\pm$ 11.9
	anti-CD4/TNF (n = 12)	91 <sup>1</sup>	102.7 $\pm$ 12.5

<sup>1</sup> Significantly reduced anti-TN3 titre ( $P < 0.005$ )

25

#### EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to specific embodiments of the invention 30 described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

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INDUSTRIAL APPLICABILITY

The invention is, inter alia, applicable to substances and preparations useful in combination therapy  
5 in the treatment of autoimmune or inflammatory diseases, such as rheumatoid arthritis in particular.

CLAIMS:

1. A combination of anti-CD4 antibody and anti-tumour necrosis factor (TNF) antibody for treating autoimmune or inflammatory diseases in a mammal, e.g. for treating rheumatoid arthritis.
2. Use of anti-CD4 antibody and anti-tumour necrosis factor (TNF) antibody for the manufacture of a therapeutic formulation for treating autoimmune or inflammatory diseases in a mammal.
3. The invention of claim 1 or claim 2, wherein the anti-CD4 antibody and the anti-TNF antibody are adapted for simultaneous or sequential administration.
4. The invention of claim 1 or claim 2, wherein the anti-CD4 antibody and the anti-TNF antibody are suitable for administration subcutaneously, intravenously or intramuscularly.
5. The invention of claim 1 or claim 2, wherein the anti-CD4 antibody and the anti-TNF antibody are in combination with a pharmaceutically acceptable vehicle.
6. The invention of claim 1, 2 or 5, in combination with an anti-inflammatory drug.
7. Use of a combination of anti-CD4 antibody and anti-TNF antibody for the manufacture of a medicament for treating rheumatoid arthritis in a mammal.

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8. A combination of an agent which affects the activation or interaction of CD4+ cells with antigen presenting cells and an inflammatory mediator, for treating autoimmune or inflammatory diseases in a mammal.  
5
9. Use of an agent which affects the activation or interaction of CD4+ cells with antigen presenting cells in combination with an inflammatory mediator,  
10 for the manufacture of a medicament for treating autoimmune or inflammatory diseases in a mammal.
10. The invention of claim 8 or 9, wherein the said agent is an antibody to T cells or to T cell receptors.  
15
11. The invention of claim 8 or claim 9, wherein the said agent is an antibody to an antigen presenting cell or to the receptors of an antibody presenting cell.
- 20 12. The invention of claim 8 or claim 9, wherein the said agent is a peptide or small molecule which blocks the T cell interaction with antigen presenting cells.
- 25 13. The invention of claim 6, or 8 to 12, wherein the inflammatory mediator is an agent capable of interfering with the activity or synthesis of TNF, or an agent capable of interfering with the activity or synthesis of either IL-1 or IL-6, or a cytokine with anti-inflammatory properties.

14. A method of treating autoimmune or inflammatory diseases in a mammal, comprising administering to said mammal a therapeutically effective amount of a combination of anti-CD4 antibody and anti-TNF antibody.  
5
15. A method of Claim 14 wherein the anti-CD4 antibody is administered simultaneously with the anti-TNF antibody.  
10
16. A method of Claim 14 wherein the anti-CD4 antibody is administered sequentially with the anti-TNF antibody.  
15
17. A method of Claim 14 wherein the anti-CD4 antibody and the anti-TNF antibody are administered subcutaneously.  
20
18. A method of Claim 14 wherein the anti-CD4 antibody and the anti-TNF antibody are administered intravenously.  
25
19. A method of Claim 14 wherein the anti-CD4 antibody and the anti-TNF antibody are administered intramuscularly.  

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20. A method of Claim 15 wherein the anti-CD4 antibody and the anti-TNF antibody are administered in a pharmaceutically acceptable vehicle.  
30
21. A method of Claim 14 wherein an anti-inflammatory drug is administered in conjunction with the anti-CD4 antibody and the anti-TNF antibody.

22. A method of treating rheumatoid arthritis in a mammal, comprising administering to said mammal a therapeutically effective amount of a combination of anti-CD4 antibody and anti-TNF antibody.
- 5 23. A method of treating autoimmune or inflammatory diseases in a mammal comprising administering to said mammal a therapeutically effective amount of a combination of an agent which affects the activation or interaction of CD4+ cells with antigen presenting cells and an inflammatory mediator.
- 10 24. A method of Claim 23 wherein the agent which affects the activation or interaction of CD4+ cells with antigen presenting cells is an antibody to T cells or to T cell receptors.
- 15 25. A method of Claim 23 wherein the agent which affects the activation or interaction of CD4+ cells with antigen presenting cells is an antibody to an antigen presenting cell or to the receptors of an antibody presenting cell.
- 20 26. A method of Claim 23 wherein the agent which affects the activation or interaction of CD4+ cells with antigen presenting cells is a peptide or small molecule which blocks the T cell interaction with antigen presenting cells.
- 25 30 27. A method of Claim 21 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of TNF.

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28. A method of Claim 21 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-1.
- 5 29. A method of Claim 21 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-6.
- 10 30. A method of Claim 21 wherein the inflammatory mediator is a cytokine with anti-inflammatory properties.
31. A method of Claim 24 wherein the inflammatory mediator is agent interfering with the activity or synthesis of TNF.
- 15 32. A method of Claim 24 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-1.
- 20 33. A method of Claim 24 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-6.
- 
34. A method of Claim 24 wherein the inflammatory mediator is a cytokine with anti-inflammatory properties.
- 25 35. A method of Claim 25 wherein the inflammatory mediator is agent interfering with the activity or synthesis of TNF.
- 30

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36. A method of Claim 25 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-1.

5 37. A method of Claim 25 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-6.

10 38. A method of Claim 25 wherein the inflammatory mediator is a cytokine with anti-inflammatory properties.

15 39. A method of Claim 26 wherein the inflammatory mediator is agent interfering with the activity or synthesis of TNF.

20 40. A method of Claim 26 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-1.

41. A method of Claim 26 wherein the inflammatory mediator is an agent interfering with the activity or synthesis of IL-6.

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25 42. A method of Claim 26 wherein the inflammatory mediator is a cytokine with anti-inflammatory properties.

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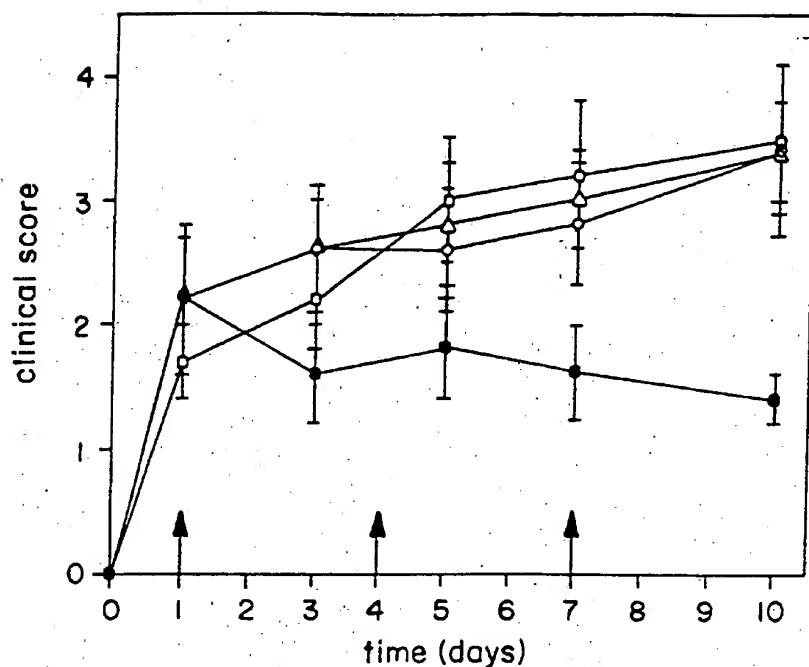


FIG. IA

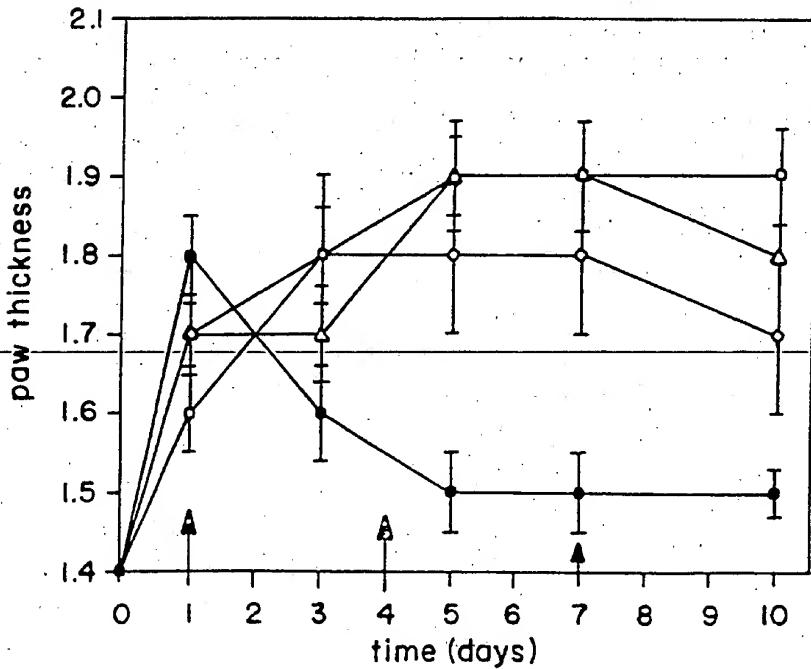
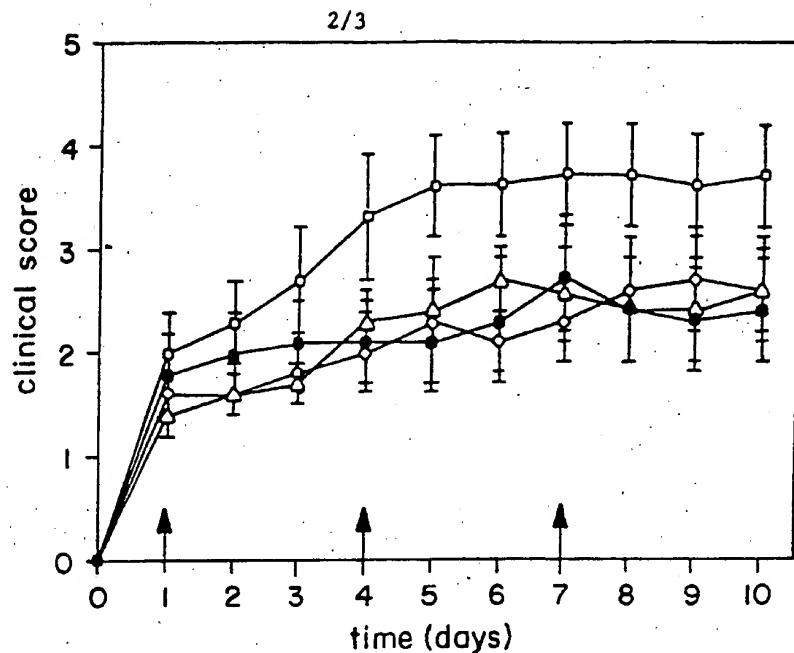
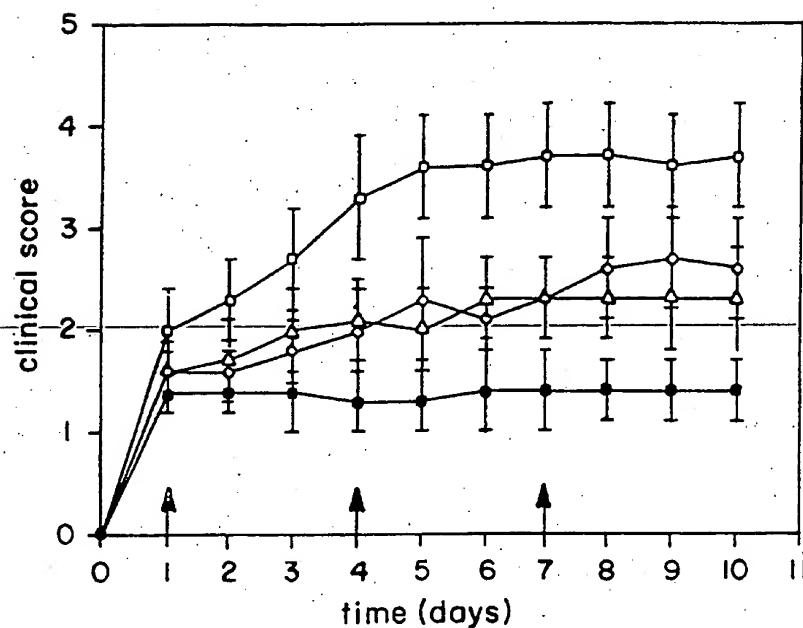
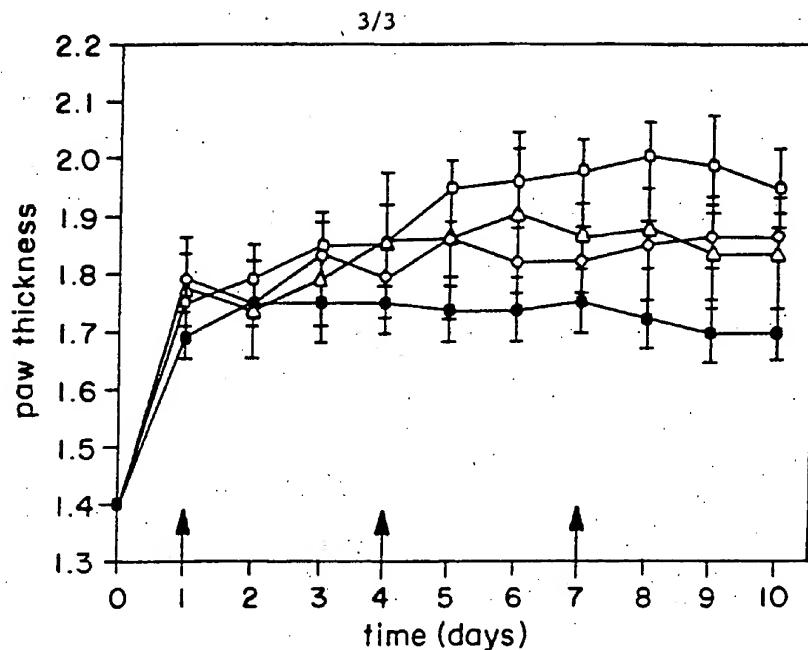


FIG. IB

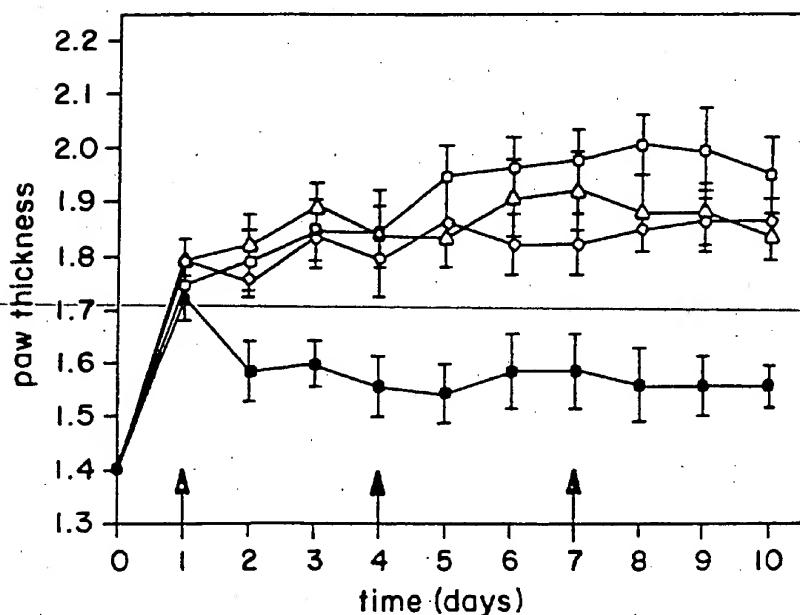
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**FIG. 2A****FIG. 2B**

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**FIG. 2C**



**FIG. 2D**

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 93/02070

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K39/395 // (A61K39/395,37:02)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>CYTOKINE vol. 3, no. 3, May 1991 pages 266 - 267 G. HORNEFF ET AL. 'Elevated levels of circulating tumor necrosis factor-alpha, interferon-gamma, and interleukin-2 in systemic reactions induced by anti-CD4 therapy in patients with rheumatoid arthritis.' see the whole document</p> <p>--- -/-</p>	1-42

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search

28 January 1994

Date of mailing of the international search report

22-02-1994

Name and mailing address of the ISA  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB93/02070

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Althoug claims 14-42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

## Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Int'l Application No.  
PCT/GB 93/02070

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE LANCET vol. 2, no. 8657 , 29 July 1989 , LONDON, GB pages 244 - 247 F. BRENNAN ET AL. 'Inhibitory effect of TNFalpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis.' see the whole document ---	1-42
Y	WO,A,89 08460 (CELLTECH LIMITED) 21 September 1989 see claims ---	1-42
Y	SCANDINAVIAN JOURNAL OF IMMUNOLOGY vol. 34, no. 5 , November 1991 , OXFORD, GB pages 627 - 633 D. STEINBRÜCHEL ET AL. 'Monoclonal antibody treatment (anti-CD4 and anti-interleukin-2 receptor) combined with cyclosporin A has a positive but not simple dose-dependent effect on rat renal allograft survival.' see abstract ---	1-42
A	CLINICAL AND EXPERIMENTAL RHEUMATOLOGY vol. 10, no. 4 , July 1992 , PISA, ITALY pages 325 - 326 F. BREEDVELD ET AL. 'Anti-CD4 antibodies in rheumatoid arthritis.' see the whole document ---	1-42
A	BRITISH JOURNAL OF RHEUMATOLOGY vol. 31, no. 5 , May 1992 , LONDON, GB pages 293 - 298 F. BRENNAN ET AL. 'TNFalpha - a pivotal role in rheumatoid arthritis.' see the whole document ---	1-42
A	WO,A,92 08474 (THE NATIONAL HEART & LUNG INSTITUTE) 29 May 1992 see claims ---	1-42
A	WO,A,92 07585 (CELLTECH LIMITED ET AL.) 14 May 1992 see claims -----	1-42

## INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No

PCT/GB 93/02070

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8908460	21-09-89	EP-A-	0403558	27-12-90
		GB-A-	2233559	16-01-91
		JP-T-	3503890	29-08-91
		US-A-	5183657	02-02-93
WO-A-9208474	29-05-92	AU-A-	8910891	11-06-92
WO-A-9207585	14-05-92	AU-A-	8758391	26-05-92
		CA-A-	2094154	02-05-92
		EP-A-	0564461	13-10-93